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Examination of *Le* and *lele* Genotypes of *Glycine max* (L.) Merr. for Membrane-Bound and Buffer-Soluble Soybean Lectin[1]

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ABSTRACT

Membrane fractions from seedlings of four soybean [*Glycine max* (L.) Merr.] lines were examined by radioimmunoassay and hemagglutination assay for the 120,000 dalton soybean lectin. Two of the lines (Sooty and T-102) are genotypically *lele* and lack buffer-soluble soybean lectin; the remaining two lines (Beeson and Harosoy 63) are *Le* and produce seeds that contain the lectin (Su *et al.* 1980 Biochim. Biophys. Acta 629: 292–304). Both Triton X-100 (0.5% v/v) and nonidet P-40 (0.05% v/v) solubilized soybean lectin from membrane fractions of *Le* cotyledons. Triton X-100 interfered substantially with the assay of protein and hemagglutinating activity and was unacceptable for use in quantitative measurements. The nonidet P-40-solubilized soybean lectin from *Le* cotyledons was consistently present both in washed 13,000*g* and 82,500*g* membrane fractions, but it accounted for less than 1.5% of the total (buffer-soluble plus membrane-bound) soybean lectin. The membrane lectin was purified by the affinity chromatography procedure devised for soluble soybean lectin, and it was immunologically indistinguishable from authentic soybean lectin. Membrane fractions from *Le* cotyledons contained insignificant amounts of radioisotope-labeled soybean lectin that had been added during homogenization, and purified membrane fractions did not bind the lectin in the presence of the hapten, D-galactose. These controls make it unlikely that the membrane soybean lectin was of cytoplasmic origin. Soybean lectin and other hemagglutinins were not present in buffer-soluble or membrane fractions from *lele* cotyledons or from roots and hypocotyls of any of the lines.

The occurrence of SBL[2] in soybean seeds is controlled by the gene designated *Le*, and lines having the homozygous recessive genotype, *lele*, lack detectable SBL (16, 17, 23). Whereas seeds of only 18 of 2,664 tested soybean [*Glycine max* (L.) Merr.] lines were phenotypically *le* (17, 21), seeds of 272 of 559 accessions of *Glycine soja* Sieb. and Zucc., the wild ancestor of *G. max* (8), lacked the lectin (22). Using sensitive radioimmunoassays and hemagglutination assays (detection limit: 1.4 μg SBL/g dry weight of tissue), we were unable to detect SBL in buffer-soluble extracts of roots, hypocotyls, or cotyledons of 5 *lele* lines (Columbia, Norredo, Sooty, T-102, Wilson 5) (23). Roots and hypocotyls of lines whose seeds contained SBL were also devoid of the lectin. The possibility that these soybean tissues contained either membrane-bound forms of SBL or other buffer-soluble hemagglutinins, however, was not rigorously excluded. Recently Bowles *et al.* (5) provided evidence that Triton X-100 extracts of membranes from leaves, shoots, and roots of unspecified soybean genotypes do in fact possess exceedingly high hemagglutinating activities. These activities were not affected by monosaccharide hapten inhibitors of SBL binding. Although it was suggested that the membrane hemagglutinins were not SBL, the molecule(s) responsible for the hemagglutinating activities associated with membranes was not purified or characterized. Here we report the results of an investigation of seedlings of the soybean lines Beeson and Harosoy 63, which are *Le*, and Sooty and T-102, which are *lele*. Our objective was to determine if SBL or other hemagglutinins are present in membrane fractions from these genotypes, each of which has been examined previously for buffer-soluble lectins (17, 23).

MATERIALS AND METHODS

Soybean Tissues. Soybean seeds of the lines Harosoy 63, Sooty, and T-102 were from Dr. Theodore Hymowitz, Department of Agronomy, University of Illinois, Urbana, IL, and Beeson soybean seeds were from Dr. R. L. Bernard, United States Department of Agriculture, Urbana, IL. Seeds were planted in 40 × 60 cm trays of vermiculite, incubated either in a greenhouse or in a controlled environment chamber (12 h photoperiod with about 2,500 lux from fluorescent lamps, 28 C day and 25 C night temperature), and watered as necessary. Roots, hypocotyls, and cotyledons were dissected from seedlings that had been gently lifted from the vermiculite and washed in a stream of cold water.

Preparation of Extracts. Two methods were used to obtain solubilized membrane fractions from soybean tissues. In the first, which was described by Bowles *et al.* (5), plant tissues were ground with a chilled mortar and pestle in one volume (v/w) of cold Hepes homogenization buffer (pH 7.6), which consisted of 15% (w/v) sucrose and 5 mM $MgCl_2$ in 50 mM Hepes (Aldrich Chemical Co., Milwaukee, WI). After removal of cell debris by centrifugation at 1,000*g*, membrane pellets (100,000*g*) were prepared as described (5). In initial experiments, the cell debris and membrane pellets were each extracted with 2 ml of 0.5% (v/v) Triton X-100 (Sigma) in 10 mM Hepes containing 5% (w/v) sucrose and 5 mM $MgCl_2$ (pH 7.0) (5) using a Polytron Homogenizer (Brinkmann Instruments, Westbury, NY). In experiments where the ability of agents other than Triton X-100 to solubilize SBL was investigated, pellets were first washed 3 times with the 10 mM Hepes washing buffer (lacking Triton X-100). The resuspended pellets were then divided into two aliquots; the first aliquot was sequentially washed with 2 ml of 100 mM D-galactose, homogenized in 2 ml of 0.5 M NaCl, and washed with 2 ml of PBS (5). The second aliquot was

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[2] Abbreviations: SBL: the 120,000 dalton soybean lectin; PBS: phosphate-buffered saline; Brij 35: polyoxyethylene 23 lauryl ether; $C_{12}TABr$: dodecyltrimethylammonium bromide; $C_{16}TABr$: hexadecyltrimethylammonium bromide; NP-40: nonidet P-40; IgG: immunoglobulin G.

washed 3 times with PBS, and further divided into four equivalent portions. These were then homogenized in 2 ml of PBS containing 0.05% (v/v or w/v) of one of the following detergents (all from Sigma): Brij 35, $C_{12}TABr$, $C_{16}TABr$, or NP-40. Extracts and wash solutions were dialyzed against PBS at 4 C and stored at −17 C.

Membrane fractions also were prepared by a modification of the procedure of Travis and Woods (24). Plant tissues were ground with a chilled mortar and pestle in two volumes (v/w) of Tris-Hepes homogenization buffer, which consisted of 25 mM Tris, 3 mM EDTA, 250 mM sucrose, 50 mM D-galactose, and 0.63 mM phenylmethylsulfonyl fluoride (Sigma) titrated to pH 7.2 with Hepes. The homogenates were filtered through Miracloth and centrifuged at 13,000*g* for 15 min. The supernatants then were centrifuged for 35 min at 82,500*g* and collected. The 13,000*g* and 82,500*g* pellets, after three washes each with PBS diluted to one-tenth strength with water, separately were extracted for 30 s with 2 ml of 0.05% (v/v) NP-40 in PBS using a Polytron Homogenizer. The detergent extracts and the 82,500*g* supernatant solutions were dialyzed against PBS and frozen at −17 C.

Analytical Methods. Hemagglutination assays of 25-μl samples of extracts were performed in polystyrene hemagglutination plates (Dynatech Laboratories, Alexandria, VA) according to standard procedures (18, 19). Controls for the biochemical specificity of hemagglutination were done by assessing inhibition by 100 mM D-galactose. Outdated human red blood cells were from the local blood bank, and chicken, horse, goat, rabbit, and sheep erythrocytes were from Pel-Freez Biologicals, Rogers, AR. Erythrocytes were washed 4 times with PBS, made to 3% (v/v) in PBS, fixed with glutaraldehyde (25) and used directly, or they were treated with trypsin (Sigma Type III) or neuraminidase (Calbiochem-Behring, La Jolla, CA) prior to fixation (19). Hemagglutination titer was defined as the reciprocal of the last dilution giving visible hemagglutination after 3 h at room temperature, and the number of hemagglutinating units in an extract was calculated by multiplying the titer of a 25-μl sample by the number of 25-μl samples contained in the total extract.

The radioimmunoassays were run as described and employed the same batch of rabbit anti-SBL IgG used earlier (23). Protein was determined by the method of Lowry *et al.* (14) using BSA as the standard.

Adherence of Radioisotope-Labeled SBL to Membranes. SBL was labeled with sodium ^{3}H-borohydride (ICN Radiochemicals, Irvine, CA), and the ^{3}H-SBL repurified by affinity chromatography as described (2, 13). Binding of soluble SBL to membranes during extraction was evaluated by adding 180 μg of ^{3}H-SBL (1.6×10^6 cpm) to from 30.2 to 35.4 g fresh weight of 7-day-old Beeson cotyledons during homogenization in 25 mM Tris-Hepes buffer. Membranes were prepared and extracted with NP-40 as described above. After centrifugation of the membrane extracts at the appropriate velocity (13,000*g* or 82,500*g*), soluble ^{3}H-SBL was determined by counting 0.5 ml samples using Ready-Solv EP and an LS-150 liquid scintillation spectrometer (both from Beckman Instruments, Palo Alto, CA). Quenching correction factors, which were obtained by counting known amounts of ^{3}H-SBL in the presence or absence of unlabeled extracts, were applied to the data. Protein was determined after dialysis of membrane extracts against PBS.

The binding of ^{3}H-SBL to membranes from 34.1 to 39.1 g fresh weight of 7-day-old Beeson cotyledons was also determined. Membranes were obtained from tissues which were homogenized in Tris-Hepes buffer, washed, and extracted with NP-40 as described above. Each membrane fraction was suspended in 10 ml of PBS prior to assay. Conical 15-ml and round-bottom 9-ml polycarbonate centrifuge tubes that had been pretreated with Prosil-28 (PCR Research Chemicals, Gainesville, FL) were used for the 13,000*g* and 82,500*g* membrane fractions, respectively. One-ml portions of the suspended membranes were dispensed into each of four tubes. Two of these tubes received 0.5 ml of PBS, and two received 0.5 ml of a hapten inhibitor, 165 mM D-galactose. PBS replaced the suspended membranes in controls for the adherence of ^{3}H-SBL to the tubes. Fifteen min after the tubes were prepared, 0.15 ml of ^{3}H-SBL in PBS (136,000 cpm and 15 μg protein) was dispensed into each. The tubes were swirled briefly, incubated at 4 C for 60 min, and centrifuged at the appropriate velocity. Residual radioactivity in the supernatant solutions was determined by counting 10,000 counts (95% confidence interval) as described earlier.

Purification and Assay of SBL from Membrane Fractions. Seven-day-old Beeson cotyledons (74.8–85.0 g fresh weight) were homogenized in 25 mM Tris-Hepes buffer, and the 13,000*g* and 82,500*g* membrane fractions were prepared and extracted with NP-40. Each extract was stirred for 1 h at 4 C with 4 to 5 ml of virgin Sepharose-*N*-caproylgalactosamine affinity adsorbent (1). Each adsorbent was washed on a sintered glass funnel with PBS, poured into a small column, and monitored at 280 nm using a cell with a 1-cm light path. Elution of the columns with 100 mM D-galactose was as described for soluble SBL (2). Affinity-purified membrane lectins were compared to affinity-purified seed lectin from the soybean variety Disoy and to affinity-purified soluble SBL from 7-day-old Beeson cotyledons by the conventional Ouchterlony double-diffusion technique. The medium was 0.75% ME Agarose (Sea-Kem, Rockland, ME), and the antibody was rabbit anti-SBL IgG. Normal serum IgG from rabbit served as the control.

RESULTS

Examination of Tissues for SBL and Other Hemagglutinins. Buffer-soluble and membrane extracts prepared from 5-day-old Beeson and 5- and 14-day-old Harosoy 63 cotyledons by the method of Bowles *et al.* (5) agglutinated native, trypsinized, and neuraminidase-treated rabbit erythrocytes and trypsinized and neuraminidase-treated human erythrocytes. Cells from goat, horse, chicken, and sheep, irrespective of enzyme treatment, were not agglutinated by these extracts. Similar extracts from Harosoy 63 and Beeson hypocotyls and roots and from all tissues of 5-day-old Sooty seedlings were completely devoid of hemagglutinating activity. Standard SBL solutions (1 mg/ml of PBS) gave titers of 32 to 1,024 in the assays, and they exhibited the same spectrum of hemagglutinating activity as did the Beeson and Harosoy cotyledon extracts. This provided initial evidence that cotyledons of *Le* lines contained both buffer-soluble and membrane SBL.

Fractions obtained from 14-day-old *Le* seedlings by the method of Bowles *et al.* (5) were chosen for systematic examination of hemagglutinating activity. Although activity was present in cell debris and membranes from cotyledons of both lines, a relatively larger fraction of the Beeson SBL was particulate (Table I). Hemagglutination was invariably prevented by the hapten, D-galactose. The activity thus was probably due to SBL, although the data do not discount the possibility that a similar D-galactose-specific lectin was present. Root and hypocotyl samples lacked hemagglutinating activity (detection limit: 0.5–1 μg SBL/g fresh weight). The Bowles *et al.* (5) extraction method, however, was unsatisfactory for quantitative measurements of membrane hemagglutinins. Triton X-100 was not removed by dialysis, and at the recommended concentration, it interfered substantially with the assay of protein. The presence of the detergent in control hemagglutination assays also caused a variable reduction in the hemagglutination titer. Thus, specific activities as hemagglutination units/mg protein could not be determined, and the relative distribution of the total hemagglutinating activity between particulate and soluble locations could not be assigned with confidence.

Additional procedures recommended by Bowles *et al.* (5) for the solubilization of membrane lectins were examined, and detergents other than Triton X-100 were evaluated. In each case, membrane pellets were washed 3 times prior to extraction to

Table I. *Hemagglutinating Activity of Buffer-Soluble Fractions and Detergent Extracts of Cell Debris and Membranes from Beeson and Harosoy 63 Cotyledons*

Cotyledons (13.7–22.0 g fresh weight) from 14-day-old plants were homogenized in 50 mM Hepes buffer. Particular fractions, obtained as described under "Materials and Methods," were extracted with 0.5% (v/v) Triton X-100, and hemagglutinating activity of extracts was determined with trypsinized human erythrocytes. The data are from replicate experiments.

Fraction	HU[a]/g fresh wt Cotyledon	% of Total HU
Beeson cell debris	750 ± 180	10 ± 3
Beeson membranes	740 ± 200	10 ± 3
Beeson buffer-soluble	6,200 ± 500	80 ± 6
Harosoy 63 cell debris	300 ± 10	3 ± 1
Harosoy 63 membranes	40 ± 0	<1
Harosoy 63 buffer-soluble	15,700 ± 7,400	97 ± 1

[a] HU = hemagglutination units, defined as the titer of a 25-μl aliquot multiplied by the number of 25-μl aliquots in the total sample.

Table II. *Soybean Lectin Extracted by Various Agents from Washed Membranes of 5-Day-Old Beeson Cotyledons*

Tissues (20 g fresh weight) were homogenized in 50 mM Hepes buffer, and a 100,000 *g* membrane fraction was prepared and washed as described under "Materials and Methods." The membranes were then divided into aliquots and washed or extracted as described below. Soybean lectin was quantified by radioimmunoassay and protein by the Folin phenol method. The results are from replicate experiments.

Extraction Agent	Treatment Prior to Extraction	Solubilized Protein	Solubilized SBL
		μg/g fresh wt	
Buffer wash	3 buffer washes	15 ± 9	0.4 ± 0.3
Buffer wash	4 buffer washes	27 ± 20	0.6 ± 0.5
Buffer wash	5 buffer washes	9 ± 1	0.3 ± 0.2
Brij 35	6 buffer washes	264 ± 50	3.7 ± 0.4
C_{12}TABr	6 buffer washes	213 ± 21	1.0 ± 1.0
C_{16}TABr	6 buffer washes	199 ± 5	1.0 ± 1.0
NP-40	6 buffer washes	355 ± 95	4.6 ± 0.9
D-Galactose wash	3 buffer washes	21 ± 2	0.6 ± 0.1
0.5 M NaCl	3 buffer washes, then D-Gal wash	46 ± 3	0.4 ± 0.1
PBS wash	3 buffer washes, then D-gal, then NaCl	49 ± 3	0.8 ± 0.1

remove residual cytoplasmic SBL. Results of replicate experiments employing radioimmunoassays to detect SBL in 5-day-old Beeson cotyledons are in Table II. All extraction agents released protein from membranes, but PBS and D-galactose washes and homogenization with NaCl were no more effective than buffer washes in solubilizing the lectin. Although the detergents were uniformly superior for protein solubilization, only Brij 35 and NP-40 consistently released relatively large amounts of SBL. At the concentration used, neither detergent interfered with protein measurement or with hemagglutination. The amount of released SBL, however, was invariably less than 1.5% of the total solubilized protein. Each of the agents also solubilized protein from membrane fractions of roots and hypocotyls, but none released detectable SBL from these tissues.

A series of experiments employing 25 mM Tris-Hepes buffer containing a protease inhibitor and a hapten provided additional information on membrane lectins. NP-40 solubilized large amounts of protein from all cotyledon samples, but only the *Le* line contained hemagglutinating activity (Table III). Approximately equivalent specific hemagglutinating activities were detected in the 82,500*g* membrane fraction and in the 13,000*g* membrane fraction, but greater than 99% of the total activity was soluble. In contrast, hemagglutinins were not detected in root and hypocotyl extracts.

Table III. *Specific Hemagglutinating Activities of Buffer-soluble Fractions and NP-40 Membrane Extracts from 7-Day-Old Soybean Cotyledons*

Cotyledons (5.0 to 28.6 g fresh weight) were homogenized in 25 mM Tris-Hepes buffer, and membrane fractions were prepared and washed as described under "Materials and Methods." Washed membrane fractions were extracted with 0.05% (v/v) NP-40, and the extracts were dialyzed against PBS prior to hemagglutination assays and protein measurement. The data are from replicate experiments.

Fraction	HU[a]/fresh wt Cotyledon	Protein/Cotyledon	Specific Activity
		mg/g fresh wt	*HU/mg protein*
13,000*g* Membranes (Harosoy 63)	240 ± 160	1.3 ± 0.8	200 ± 100
82,500*g* Membranes (Harosoy 63)	110 ± 60	0.8 ± 0.3	140 ± 30
82,500*g* Soluble (Harosoy 63)	59,200 ± 19,000	35.0 ± 10.3	1,670 ± 80
13,000*g* Membranes (T-102)	0	1.8 ± 1.3	
82,500*g* Membranes (T-102)	0	3.0 ± 0.5	
82,500*g* Soluble (T-102)	0	52.8 ± 16.8	

[a] HU = hemagglutination units, defined as the titer of a 25-μl aliquot multiplied by the number of 25-μl aliquots in the total sample. Hemagglutination in the presence of 100 mM D-galactose was not observed.

Table IV. *Adherence of ^{3}H-SBL to Suspended Membranes from 7-Day-Old Beeson Cotyledons*

Tissues were homogenized in 25 mM Tris-Hepes buffer, and membranes were prepared and washed 3 times as described under "Materials and Methods." The membranes were then suspended in PBS and dispensed into tubes containing PBS or D-galactose in PBS. Tubes with PBS replacing membranes served as controls for the nonspecific binding of ^{3}H-SBL to the tubes. After 15 min, 15 μg of ^{3}H-SBL was added to each tube, and 1 h later the tubes were centrifuged and soluble radioactivity determined. The data are from replicate experiments.

Sample	Protein[a] (mg/sample)	% of Added SBL Soluble in the Presence of	
		PBS	Galactose
13,000*g* Membranes	3.1 ± 0.1	84 ± 2	102 ± 1
PBS control		93 ± 6	100 ± 2
82,500*g* Membranes	4.1 ± 1.3	99 ± 1	102 ± 4
PBS control		97 ± 1	101 ± 2

[a] Solubilized by 0.05% NP-40.

Adherence of Exogenous SBL to Membrane Fractions. Because Beeson and Harosoy 63 cotyledons contained large amounts of buffer-soluble SBL, two series of experiments were performed to examine the possibility that, precautions notwithstanding, the membrane SBL was of cytoplasmic origin. In the first experiment, 7-day-old Beeson cotyledons were homogenized in the presence of 180 μg of ^{3}H-SBL, and NP-40 extracts of membrane fractions were examined for radioactivity. Membrane extracts from replicate experiments, after correction for quenching, contained from 34 to 252 cpm/ml extract. These values are essentially background levels, and the maximum amount of radioactivity released by NP-40 was only 500 cpm (equivalent to 56 ng of ^{3}H-SBL contained in

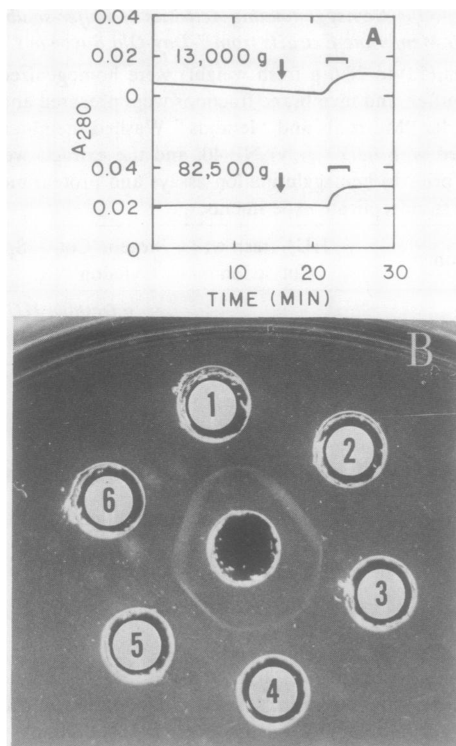

FIG. 1. Affinity chromatography of membrane SBL from 7-day-old Beeson cotyledons and detection of the lectin by immunodiffusion. A, absorbance (280 nm) of eluates from affinity columns. The 13,000g and 82,500g membrane fractions were prepared and solubilized with NP-40 as described under "Materials and Methods." The arrows mark the point at which elution of each column with 100 mM D-galactose was initiated, and the brackets indicate the batch of SBL collected for analysis. B, immunodiffusion. The center well contained 20 μl of rabbit anti-SBL IgG, and the peripheral wells contained 20 μl volumes of affinity-purified SBL from the following sources: (1) the 82,500g membrane SBL; (2) buffer-soluble SBL from 7-day-old Beeson cotyledons; (3) the 82,500g membrane SBL; (4) the 13,000g membrane SBL; (5) Disoy seed SBL; (6) buffer-soluble SBL from 7-day-old Beeson cotyledons. Normal rabbit serum IgG did not form a precipitate with any of the antigens.

3.5 mg of solubilized protein from the 82,500g fraction).

The binding of ^{3}H-SBL to washed membrane fractions is given in Table IV. In the presence of the hapten, which was routinely included in the Tris-Hepes homogenization buffer, purified SBL did not bind to membranes. In the absence of the hapten, a small amount of radioactivity (equivalent to 2.6 ng SBL/mg NP-40-solubilized protein) adhered to the 13,000g membranes, but none adhered to the 82,500g membranes.

Purification of Membrane SBL. The lectin solubilized from 13,000g and 82,500g membranes from 7-day-old Beeson cotyledons was purified by affinity chromatography. Since the amount of solubilized lectin was small, virgin affinity adsorbent was used, and precautions were taken to rigorously preclude cross-contamination of samples. A readily detectable amount of protein (A_{280}) was specifically eluted from each affinity column by 100 mM D-galactose (Fig. 1). The specifically eluted protein from both the 13,000g and 82,500g membrane fractions formed an immunoprecipitate with rabbit anti-SBL IgG, and in each case the precipitate lines fused in a reaction of immunological identity with bands from authentic SBL obtained either from seeds or cotyledons (Fig. 1). Identical results were obtained with analogous membrane fractions from 7-day-old Harosy 63 cotyledons.

DISCUSSION

Hemagglutinins have been detected in the membranes of a number of organisms (15), including higher plants (3–6). Whether plant membrane hemagglutinins are related to the soluble seed lectins, and indeed whether they correctly can be termed lectins (see ref. 7), generally remains unclear. The binding specificities of the membrane hemagglutinins from peanut (*Arachis hypogaea* L.) and soybean, however, are apparently distinct from those of the soluble seed lectins of the same species (5). In the present study, screening assays employing a limited variety of erythrocytes failed to detect any hemagglutinins with activity distinct from that of SBL. Moreover, using various extraction methods and seedlings of several genotypes and ages, we failed to find SBL in membranes of roots and hypocotyls. Our results are in apparent conflict with reports by Bowles *et al.* (5) and Stacey *et al.* (20). Although the reason for the conflicts may be easily explained, *i.e.* the use of different soybean lines or seedling growth conditions, others also have had great difficulty in reproducibly obtaining hemagglutinating activity from soybean roots (K. Keegstra, personal communication). It is noteworthy that Triton X-100, when used at concentrations recommended by Bowles *et al.* (5), interfered strongly both with the Lowry protein assay and the hemagglutination assay. Such undesirable effects of Triton X-100 on lectin activity (12) and protein measurement (10) are well known, and they prevented us from obtaining reliable data from tissues extracted according to the earlier method. More difficult to explain are the data of Stacey *et al.* (20), who used an indirect immunolatex method to secure evidence that soybean root hair surfaces contain SBL. The discrepancy in the results, which should be given further attention, perhaps relates to the inability of detergents to solubilize particulate SBL, or to the leaching under some conditions of soluble SBL from seeds to root surfaces.

In our studies, the only particulate fractions containing hemagglutinating activity were from *Le* cotyledons. Evidence that SBL was responsible for the activity of membrane fractions is as follows: (a) the spectrum of membrane hemagglutinating activity against a panel of erythrocytes was the same as that of authentic SBL; (b) the crude membrane extracts contained a protein that was detected by radioimmunoassays for SBL; (c) affinity chromatography with an adsorbent designed for SBL purification purified a protein which, in double diffusion tests with rabbit anti-SBL IgG, formed a line of immunological identity with authentic SBL. An earlier study showed that in ungerminated Altona soybean seeds, SBL was localized in protein bodies but not in association with membranes (9). The membrane SBL of *Le* seedling cotyledons thus may be SBL that was mobilized from protein bodies of dry seeds.

Köhle and Kauss (11) recently demonstrated that the castor bean (*Ricinus communis* L.) agglutinin, previously thought to be a membrane component (6), was in fact a cytoplasmic contaminant. We consider it unlikely that the membrane SBL was similarly of cytoplasmic origin; little or no exogenously supplied ^{3}H-SBL adhered to membranes during extraction, and ^{3}H-SBL did not bind to purified membranes. Our data should nevertheless be interpreted with caution, until corroborating evidence is obtained that SBL is a membrane component. Although the question of the function of both buffer-soluble and membrane SBL in *Le* cotyledons remains as unfinished business, it should be emphasized that *lele* genotypes, which are devoid of the lectin, grow and reproduce in a normal fashion (17, 21–23).

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